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Increased Vesicular Monoamine Transporter Binding during Early Abstinence In Human Methamphetamine Users: Is VMAT2 a Stable Dopamine Neuron Biomarker?

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Animal data indicate that methamphetamine can damage striatal dopamine terminals. Efforts to document dopamine neuron damage in living brain of methamphetamine users have focused on the binding of [^{11}C]dihydrotetrabenazine (DTBZ), a vesicular monoamine transporter (VMAT2) positron emission tomography (PET) radioligand, as a stable dopamine neuron biomarker. Previous PET data report a slight decrease in striatal [^{11}C]DTBZ binding in human methamphetamine users after prolonged (mean, 3 years) abstinence, suggesting that the reduction would likely be substantial in early abstinence. We measured striatal VMAT2 binding in 16 recently withdrawn (mean, 19 d; range, 1–90 d) methamphetamine users and in 14 healthy matched-control subjects during a PET scan with (+)[^{11}C]DTBZ. Unexpectedly, striatal (+)[^{11}C]DTBZ binding was increased in methamphetamine users relative to controls (+22%, caudate; +12%, putamen; +11%, ventral striatum). Increased (+)[^{11}C]DTBZ binding in caudate was most marked in methamphetamine users abstinent for 1–3 d (+41%), relative to the 7–21 d (+15%) and >21 d (+9%) groups. Above-normal VMAT2 binding in some drug users suggests that any toxic effect of methamphetamine on dopamine neurons might be masked by an increased (+)[^{11}C]DTBZ binding and that VMAT2 radioligand binding might not be, as is generally assumed, a "stable" index of dopamine neuron integrity *in vivo*. One potential explanation for increased (+)[^{11}C]DTBZ binding is that VMAT2 binding is sensitive to changes in vesicular dopamine storage levels, presumably low in drug users. If correct, (+)[^{11}C]DTBZ might be a useful imaging probe to correlate changes in brain dopamine stores and behavior in users of methamphetamine.

Key words: **vesicular monoamine transporter 2; positron emission tomography; dihydrotetrabenazine; methamphetamine; dopamine; monoamine**

Introduction

Methamphetamine (MA) is a widely used stimulant drug which, preclinical animal data have shown, has the potential to damage brain dopamine (DA) containing neurons (specifically axon terminals) in human users of the drug (Seiden and Ricaurte, 1987). A new public health concern is the possibility, based on recent nonhuman primate data, that even "low" therapeutic doses of MA or amphetamine used clinically in children for treatment of attention deficit hyperactivity disorder might be sufficient to damage brain DA neurons (Ricaurte et al., 2005).

With the aim of resolving the question of MA toxicity to brain DA neurons in humans, a focus has been on the use of the vesicular monoamine transporter (VMAT2) as a marker of DA neuronal integrity. Although VMAT2 is also present in nondopaminergic neurons (serotonin, noradrenaline), most VMAT2 in the DA-rich striatum is considered to be localized to DA nerve terminals (Vander Borght et al., 1995a; Wilson et al., 1996b; Frey et al., 1997). The election of VMAT2 as the present "gold standard" DA marker is based on extensive animal data suggesting that levels of VMAT2 are resistant to drug-compensatory regulation affecting other DA markers (e.g., DA, DA transporter) (Vander Borght et al., 1995b; Kilbourn et al., 1996; Wilson et al., 1996b; Frey et al., 1997; Kemmerer et al., 2003).

In the first study of VMAT2 in brain of human MA users, striatal levels of VMAT2, as inferred from binding of dihydrotetrabenazine ([^{3}H]DTBZ), were normal despite marked loss of DA and the DA transporter (Wilson et al., 1996a). Although this unexpected finding suggested that MA might not damage DA neurons in humans, the results of this autopsied-brain study could not be definitive because of the limited sample size and the generic uncertainty whether postmortem data would translate to

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Table 1. Demographic and clinical characteristics of subjects

	Control subjects	Methamphetamine users	*p* value
Age	29.7 ± 5.4 years	27.8 ± 5.7 years	0.37
Gender	11 male; 3 female	11 male; 5 female	
Ethnicity	11 Caucasian, 2 Asian, 1 Eastern Indian	13 Caucasian, 2 Black, 1 Maghreban	
Education	15.5 ± 2 years	12.12 ± 2 years	0.0001
Premorbid IQ[a]	117.1 ± 5.8	117.1 ± 4.9	0.5
Cigarette smokers	5 smokers; 1.7 ± 3 cigarettes per day	9 smokers; 4.5 ± 3 cigarettes per day	0.07
Alcohol use	3 ± 2 drinks per week	4 ± 3 drinks per week	0.19
Years of MA use	N.A.	5.1 ± 3 years; range, 2–11 years	
Route of administration	N.A.	8 nasal, 3 smoke, 2 nasal/smoke, 2 i.v./smoke, 1 nasal/oral	
Days used: last 30 d	N.A.	5.6 ± 3 d; range, 0–10 d	
Days since last MA use	N.A.	19 ± 24 d 5, 1–3 d; 7, 7–21 d; 4, >30 d	
Positive drug screen			
On screening visit	0	8, MA/amphetamine; 3, THC; 1, cocaine; 1, MDMA	
On PET scan day[b]	1 THC	5, MA/amphetamine; 3, cocaine; 3, THC	
Other drugs used recently[c]	2 THC	4, THC; 6, MDMA/MDA; 10, cocaine; 2, opiates; 2, benzodiazepines	

MA, Methamphetamine; THC, tetrahydrocannabinol (cannabis); MDMA, 3,4-methylenedioxy-N-methlamphetamine (ecstasy); MDA, 3,4-methylenedioxyamphetamine; N.A., not applicable.

[a]Intelligence Quotient (IQ) as per results on the National Adult Reading Test (Nelson, 1982).

[b]The 5 MA-positive subjects reported using the drug 1.5 to 3 d before the scan; the 3 cocaine-positive subjects reported using the drug 2–7 d before the scan.

[c]As per scalp hair data (~4–5 months prior to the PET experiment, assuming hair grows ½ inch per month).

living human brain. This issue was finally addressed in a positron emission tomography (PET) imaging investigation of (+)[^{11}C]DTBZ in "heavy" MA users in which striatal VMAT2 binding was reported to be decreased but only by 10% (Johanson et al., 2006). Unfortunately, the design of this study did not permit easy resolution of the status of brain VMAT2 in MA users as the average time between last use of the drug and PET scan was 3 years. It could reasonably be argued that after such extended abstinence some, probably extensive, neuronal recovery had occurred (Sekine et al., 2001; Volkow et al., 2001b; Chou et al., 2007) and that had subjects been selected in early abstinence, the VMAT2 reduction would likely have been substantial.

Based on the finding of reduced striatal (+)[^{11}C]DTBZ binding in MA users 3 years into drug withdrawal, our objective was to establish by PET imaging whether striatal binding of the same radioligand would be substantially decreased in early abstinence from MA use. Contrary to our prediction, we found that striatal (+)[^{11}C]DTBZ binding was increased, an observation that we suggest is related to our original discovery of very low striatal DA in MA users (Wilson et al., 1996b; Moszczynska et al., 2004).

Materials and Methods

Subjects. Fourteen healthy subjects and 16 active MA users self-referred (in response to web advertisement) to participate in the PET imaging study. In addition, three otherwise healthy patients with early stage Parkinson's disease [two male, one female, 51.3 ± 6.1 years old; Hoehn and Yahr Scale (Hoehn and Yahr, 1967): two stage 1, one stage 2; Unified Parkinson's Disease Rating Scale (Fahn et al., 1987): 16.7 ± 1.4] were recruited to participate as a "positive" control, as we and others have shown striatal [^{11}C]DTBZ and [^{3}H]DTBZ binding to be decreased in this condition (Frey et al., 1996; Wilson et al., 1996b; Lee et al., 2000; Bohnen et al., 2006). Table 1 provides the demographic characteristics of the control and MA user samples. All subjects gave written informed consent approved by the Centre for Addiction and Mental Health Research Ethics Board and underwent a full screening interview which included (1) a psychiatric evaluation using the Semi-Structured Clinical Interview for Diagnostic and Statistical Manual of Mental Disorders (DSM)-IV axis I disorders (First et al., 1995); (2) a brief neurological assessment including the administration of the Unified Parkinson's Disease Rating Scale (Fahn et al., 1987) and the Purdue pegboard task (Lafayette Instrument Company); (3) a complete medical evaluation which included a physical exam, blood chemistry profile, complete blood count, 12 lead electrocardiogram, urinalysis (gas chromatography-mass spectrometry), scalp hair toxicology [modified from Kalasinsky et al. (2001)], and pregnancy test (repeated on the scan day); and (4) the administration of mood [Beck depression inventory (BDI) (Beck et al., 1961); Inventory of Depressive Symptomatology (IDS) (Rush et al., 2000)] and drug use questionnaires (locally developed). All subjects were males or nonpregnant, nonlactating females between the ages of 18 and 45 years old; all were free of significant medical conditions and of current or personal history of DSM-IV Axis I disorders (excluding MA abuse/dependence in MA users and nicotine dependence). Study criteria for active MA use included the following: (1) meeting DSM-IV criteria for MA abuse or dependence; (2) test positive for MA in hair; (3) no current (12 months) abuse or dependence of drugs other than MA (except nicotine).

Radiochemical synthesis of (+)[^{11}C]DTBZ. (+)[^{11}C]DTBZ was prepared by modification of the method described by the Michigan group (Jewett et al., 1997). Briefly, [^{11}C]-iodomethane was reacted with (+)-9-O-desmethyl-α-dihydrotetrabenazine in the presence of base using the "loop" method (Wilson et al., 2000), purified by HPLC, and formulated as a sterile pyrogen-free saline solution. Radiochemical purities were >98%.

Image acquisition protocol. PET images were acquired using the second-generation high-resolution CPS-HRRT neuro-PET camera system (Siemens Medical Imaging; in-plane resolution of ~2.8 mm, full-width at half-maximum). On the day of the scan, subjects were asked to provide a urine sample for drug toxicology (and pregnancy test in females); a urine sample positive for drug use on the day of testing was not grounds for exclusion in MA subjects. MA craving was assessed with the Desire for Speed Questionnaire [(DFS) (James et al., 2004); desire to use; intention to use; anticipation of positive outcome; anticipation of relief from withdrawal or distressing symptoms], and last day of drug use was recorded. Cognitive testing was conducted on a different day (MA users were >7 d abstinent). Patients with Parkinson's disease were scanned in a drug-free state (two were drug-naive patients; one was 12 h withdrawn from L-dopa + pergolide). Subjects were studied supine with their head held in place using a custom-made thermoplastic facemask fixation system (Tru Scan Imaging). Transmission scans obtained using a single photon ^{137}Cesium (Eγ = 662 keV) point source and used to correct the emission scans for the attenuation of 511 keV photons through tissue and head support. The scan was initiated after the bolus injection, in the antecubital vein, of a 358 ± 40 MBq (9.68 ± 0.55 mCi) of (+)[^{11}C]DTBZ. The average injected mass was 4.54 μg with an average specific activity corresponding to 1073 mCi/μm. The emission data were acquired for 90 min in 32-bit list mode (19 frames: 4 × 1 min, 3 × 2 min,

8 × 5 min, and 4 × 10 min frames). Raw data were reconstructed by the Fourier rebinning two-dimensional (2D) filtered-back projection algorithms (Defrise et al., 1997). Proton-density magnetic resonance images (MRI) (repetition time, 6000 ms; echo time, 17 ms; slice thickness, 2 mm and zero gap; 85 slices; field of view, 22 cm × 16 cm; 256 × 256, yielding a voxel size of 1.5 mm × 0.86 mm × 0.86 mm slice thickness of 2 mm; number of excitation, 2) were obtained on a General Electric Medical System Signa 1.5T MRI scanner (General Electric Medical Systems).

Region of interest analysis of the PET data. Region of interest (ROI) delineation and analysis was performed by using in-house software (ROMI); details of ROI delineation are described by Rusjan et al. (2006). In brief, a standard brain template (International Consortium for Brain Mapping/Montreal Neurological Institute 152 MRI) containing a set of predefined cortical and subcortical ROIs [based on Talairach et al. (1988) and Kabani et al. (1998) atlases] was nonlinearly transformed [statistical parametric mapping (SPM) normalization and coregistration; Wellcome Department of Cognitive Neurology, London, UK; http://www.fil.ion.ucl.ac.uk/spm/] to fit individual high-resolution MRI. Each individual's set of automatically created ROIs was then refined by iteratively including and deleting voxels based on the probability of each voxel belonging to gray matter (SPM2 segmentation, Wellcome Department of Cognitive Neurology, London, UK; http://www.fil.ion.ucl.ac.uk/spm/). Each individual's refined ROIs were aligned and resliced to match the dimension of the PET images (normalized mutual information algorithm implemented under SPM2) (Studholme et al., 1999). Three bilateral sub compartments of the striatum were selected as ROIs using the criteria outlined in the literature (Martinez et al., 2003). These ROIs correspond to left and right dorsal caudate (DC), dorsal putamen (DP), and ventral striatum (VS). The whole thalamus and the midbrain were also investigated. The occipital cortex was selected as the region of reference (i.e., devoid of significant levels of VMAT2) (Scherman et al., 1988). (+)[C11]DTBZ binding to VMAT2 was estimated in each ROI using the simplified reference tissue method (SRTM) (Lammertsma and Hume, 1996; Gunn et al., 1997) and the occipital cortex time activity curve as an input function (Koeppe et al., 1996; Chan et al., 1999). This operation was performed with PMOD (version 2.8.5; PMOD Technologies). The outcome measure derived from this analysis is BP_{ND} (the specific to nonspecific partition coefficient, commonly termed as non-displaceable binding potential). BP_{ND} is equal to B_{max}/K_D where B_{max} is unoccupied VMAT2 density, K_D is the *in vivo* affinity of (+)[11C]DTBZ. The SRTM has been shown to be an appropriate model for quantifying (+)[C11]DTBZ data in humans without arterial input function (Koeppe et al., 1996; Chan et al., 1999). For comparison, we also derived (+)[11C]DTBZ BP_{ND} using the graphical approach described by Logan et al. (1990).

Voxelwise analysis of (+)[11C]DTBZ binding. Parametric images of (+)[11C]DTBZ binding were generated by estimating SRTM parameters by conventional weighted linear regression using operational equations of integral form (Zhou et al., 2003). The tissue time activity curve of the occipital reference region served as input function. The application of the model was performed on the 3D dyadic wavelet transformed (3D-DWT) dynamical PET image using ROMI (Rusjan et al., 2006). This approach has been shown to overcome noise susceptibility when solving linear models in the real-space and to be reliable across regions of different receptor density in the presence of noise (Turkheimer et al., 1999; Cselényi et al., 2002, 2006). Each parametric map was spatially normalized to an anatomical template (Montreal Neurological Institute) using SPM2 normalization and coregistration tools (Wellcome Department of Cognitive Neurology, London, UK; http://www.fil.ion.ucl.ac.uk/spm). Once in the same space BP_{ND} maps were statistically investigated to assess significant contrasts between groups using independent sample *t* test analysis (SPM5).

Statistical analyses. Comparisons between (+)[11C]DTBZ VMAT2 BP_{ND} in the different ROIs in the MA users and control subjects were conducted by using a repeated-measures ANOVA (Statistical Program for the Social Sciences 15) with a within-subject factor (ROI) and a between-subject factor (MA users and control groups). When appropriate, least significant difference *t* tests, Bonferroni corrected for planned comparison, were applied to determine the significance of regional differences in (+)[11C]DTBZ BP_{ND} between groups. Pearson product moment correlation coefficients (and Spearman's ρ) were used to examine the putative relationships between MA use (frequency in the last 30 d, years of use, days of abstinence), regional (+)[11C]DTBZ BP_{ND}, and mood and cognitive status.

Results

Demographics and subjects characteristics

MA use and recency of use were confirmed by corroborating self-report drug-history, urinalysis, scalp (14 of 16) or chest hair (1 of 16) analysis (Table 1). One scalp hair sample in an MA user was too short to provide >1 month history; however, strong evidence suggested a chronic history of MA use. A drug-free history (except cannabis use) was confirmed in all control subjects by scalp hair and urinalysis. Scores on depressive inventories were significantly greater in MA users relative to controls (BDI: control 1.64 ± 1.4 vs MA users 6.2 ± 6.7, $p = 0.02$; IDS: control 3.4 ± 3.8 vs MA users 10.3 ± 8.5, $p = 0.01$) and correlated positively with frequency of MA use (BDI and MA use in the last 30 d: $r = 0.59$; $p = 0.017$).

Neuropsychological, neurological, and cognitive testing

Results of a battery of neurological and neuropsychological tasks revealed mild impairment in tests of attention, psychomotor speed, and decision making in MA users relative to control subjects. MA users were slower during the pegboard task of motor dexterity (Lafayette Instrument Company, 1985) (dominant hand: controls 15.6 ± 2.0 vs MA users 14.1 ± 1.4 pegs; $p = 0.03$) and performed below controls on the perceptual speed component of the Trail Making Test (Reitan, 1958) (parts A: controls 21.7 ± 4.9 s vs users 25.3 ± 4.6 s; $p = 0.04$). They had a small, although significant memory deficit after delay in the cued-recall portion of the California Verbal Learning Test (controls 14.06 ± 1.7 vs MA users 12.6 ± 2.2 items; $p = 0.03$) (Delis et al., 1987). The MA users also displayed slightly below normal (nonsignificant) working memory as revealed by the Trail Making Test parts B (Reitan, 1958) (controls 45.6 ± 10.2 s vs MA users 53.5 ± 10.7 s; $p = 0.12$) and digit span subtest of the Wechsler Memory Scale-Revised (Wechsler, 1981) (control 75.4 ± 17 items vs MA users 64.6 ± 17 items; $p = 0.11$). The Iowa Gambling Task revealed a significantly impaired decision-making strategy in MA users relative to controls (Bechara et al., 1994) ($p = 0.0004$).

Voxelwise analysis of (+)[11C]DTBZ BP_{ND}

Voxelwise analysis over a brain volume corresponding to the striatum, globus pallidus, thalamus, and midbrain did not reveal that MA use was associated with decreases in (+)[11C]DTBZ BP_{ND}, but rather the opposite. Small increases in (+)[11C]DTBZ BP_{ND} were found in MA users relative to controls. Peak clusters occurred bilaterally in the DP (right: MNI coordinates, 22, −2, −12, *t* max = 3.56, $k = 76$, *p* uncorrected = 0.001; left: MNI coordinates, −20, 15, 0, *t* max = 2.73; $k = 44$; *p* uncorrected = 0.005) and in the head of the DC (right: MNI coordinates, 14, 18, 0, *t* max = 2.68, $k = 82$, *p* uncorrected = 0.006; left: MNI coordinates, −12, 16, −4, *t* max = 2.10, $k = 7$, *p* uncorrected = 0.023). On the scan day, five MA users tested positive for MA in urine and reported using the drug 1.5–3 d before. When investigating users in very early abstinence (1–3 d) relative to control subjects, larger clusters of significant contiguous voxels were found. These clusters occurred in the right (MNI coordinates, 25, 5, −4, *t* max = 6.63, $k = 360$, *p* false discovery rate correction = 0.014) and left (MNI coordinates, −16, 6, 6, *t* max = 4.99, $k = 236$, *p* false discovery rate correction = 0.04) DP and extended to

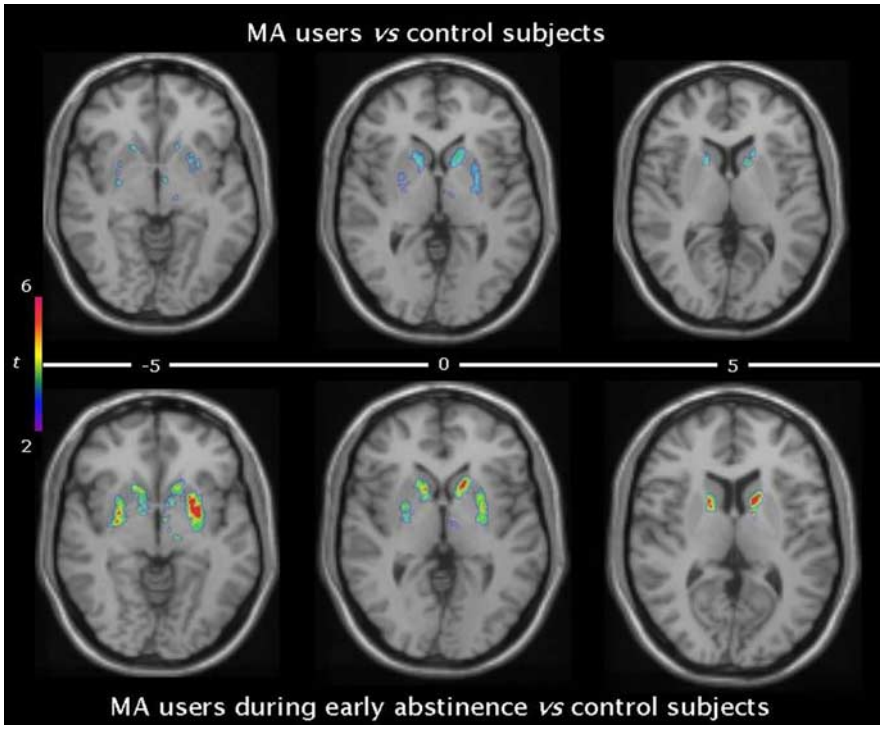

Figure 1. *t* Statistical map of (+)[^{11}C]DTBZ BP_{ND} change illustrating increased (+)[^{11}C]DTBZ BP_{ND} in (top) all MA users and (bottom) in MA users during very early abstinence (1–3 d) relative to matched control subjects. Colored *t* maps are overlaid on an average T1 MRI. MNI coordinates: $z = -5$, 0, and 5.

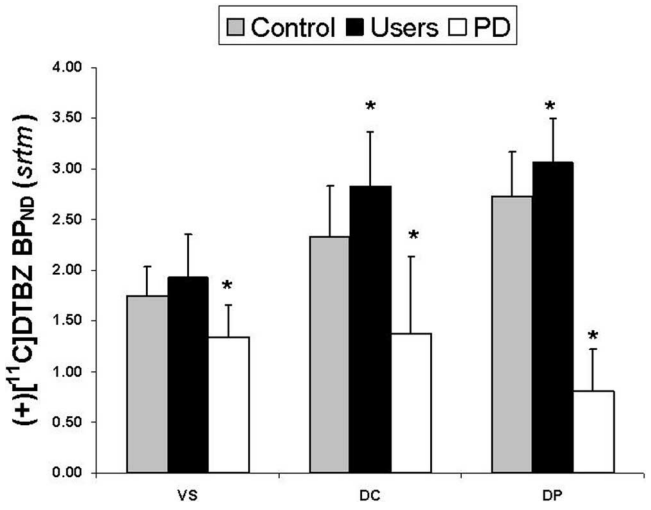

Figure 2. Mean (SD) (+)[^{11}C]DTBZ BP_{ND} in three subcompartments of the striatum in MA users, matched controls, and patients with Parkinson's disease (*$p < 0.05$, significantly different from control).

the lateral and medial globus pallidus and to the head of the DC bilaterally (Fig. 1).

Region of interest analysis of (+)[^{11}C]DTBZ BP_{ND}

ROI analyses confirmed the results obtained on the voxelwise search. The ANOVA indicated a significant region-specific increase in (+)[^{11}C]DTBZ BP_{ND} (ROI × group: $F_{(4, 112)} = 4.171$; $p = 0.003$) in MA users relative to controls (Fig. 2). This increase corresponded to 22% ($p = 0.012$) in the DC, 12% ($p = 0.035$) in the DP, and 11% [not significant (N.S.)] in the VS. The (+)[^{11}C]DTBZ BP_{ND} in the patients with Parkinson's disease ($n = 3$) was significantly reduced in all regions (−24 to −70%), relative to controls, with, as expected (Kish et al., 1988; Frey et al., 1996; Bohnen et al., 2006; Martin et al., 2008), the most marked reduction occurring in the DP (−70%). We investigated whether time since last MA use (Table 1) influenced (+)[^{11}C]DTBZ BP_{ND}. An ANOVA using time since last MA use as a grouping variable (1–3, 7–21, >30 d) and ROI as a repeated factor (ROI × time since last MA use: $F_{(12, 104)} = 2.80$; $p = 0.002$) indicated that (+)[^{11}C]DTBZ BP_{ND} was significantly increased in the DC (41%; $p = 0.001$), DP (27%; $p = 0.003$), and VS (35%; $p < 0.0005$) of subjects who had recently used MA (1–3 d) relative to controls. Subjects who used MA between 7 and 21 d before the PET scan also showed a regional increase in (+)[^{11}C]DTBZ BP_{ND} (DC 15%, DP 8%; N.S.); however, this effect was not significant (relative to controls). Subjects who had been abstinent for >30 d had (+)[^{11}C]DTBZ BP_{ND} only very slightly above control range (DC 9%, DP 4%; N.S.). Visual inspection of striatal (+)[^{11}C]DTBZ BP_{ND} revealed that all but four MA users had (+)[^{11}C]DTBZ BP_{ND} values within the top half of the control range, highest (+)[^{11}C]DTBZ BP_{ND} were observed during early abstinence and lowest (+)[C^{11}]DTBZ BP_{ND} were found in the three Parkinson's cases (Fig. 3). The length of the drug-free interval (1–3, 7–21, >30 d) correlated with (+)[^{11}C]DTBZ BP_{ND} in all subcompartments of the striatum such that shorter drug-free interval were associated with greater (+)[^{11}C]DTBZ BP_{ND} (Spearman's *r*: DC, −0.6, $p = 0.02$; DP, −0.5, $p = 0.04$; VS, $= -0.7$, $p = 0.003$). Drug craving (DFS), severity of dependence, and frequency of use in the last 30 d did not correlate with (+)[^{11}C]DTBZ BP_{ND}.

(+)[^{11}C]DTBZ was also estimated using the Logan graphical method (Logan et al., 1990). A good agreement between BP_{ND} estimates from both methods was found, although Logan derived BP_{ND} estimates were slightly lower than SRTM values (4–7% in striatal subcompartments). The correlation coefficient between the BP_{ND} estimates from each method was high ($r = 0.91$–0.97 in striatal subcompartments). The two methods were found to yield similar results (ROI × group ANOVA: $F_{(4, 112)} = 2.646$, $p = 0.037$; DC, 18.5%, $p = 0.048$; DP, 11.3, $p = 0.062$), although Logan-derived BP_{ND} had slightly higher variability.

Discussion

Contrary to our prediction, we found that MA use was not associated with decreased but rather with increased (+)[^{11}C]DTBZ binding, particularly during acute drug withdrawal. A finding of increased (+)[^{11}C]DTBZ binding in a human condition is, with one exception (see below), unprecedented in the literature and questions the validity of (+)[^{11}C]DTBZ/VMAT2 binding as a stable index of DA neuron integrity.

Striatal VMAT2 binding is not decreased in chronic MA users

Our failure to detect reduced (+)[^{11}C]DTBZ BP_{ND} in MA users, a presumed reliable measure of DA terminals, raises the possibility, initially suggested by our postmortem data (Wilson et al.,

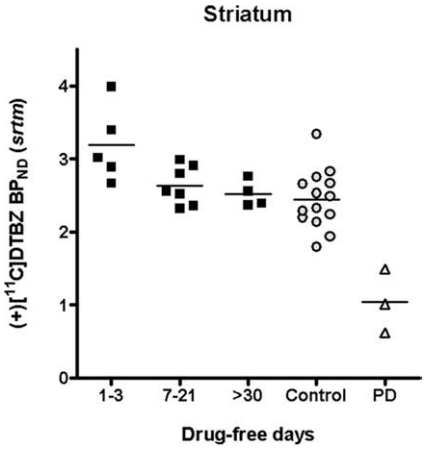

Figure 3. Scatter plot of (+)[11C]DTBZ BP_{ND} in whole striatum of MA users at different stages of abstinence (1–3, 7–21, >30 d) in matched controls and in patients with Parkinson's disease.

1996b), that self-administered MA might not be toxic to DA neurons in humans, as is the case in animals (Seiden and Ricaurte, 1987). Another possibility is that MA does in fact damage DA neurons in the human but that the MA users tested in the present study were not using sufficient amounts of the drug to cause toxicity. In this regard, the MA users of our study were using somewhat less MA than previously reported by others (Johanson et al., 2006). Nonetheless, the subjects displayed similar patterns of mild cognitive and neurological impairment, depressed mood/anhedonia, and increased agitation/restlessness as reported in studies of "heavy" users (Volkow et al., 2001a; Chang et al., 2002; Johanson et al., 2006). Alternatively, as discussed below, we might not have found decreased (+)[11C]DTBZ binding in the MA users because an apparent change in VMAT2 availability (by unknown mechanisms) masked a possible decrease in VMAT2 levels.

MA use increases striatal (+)[11C]DTBZ binding-possible mechanisms

There are several technical/artifactual issues which might explain elevated (+)[11C]DTBZ in MA users. Increased (+)[11C]DTBZ binding might reflect structural changes in brain of MA users which might influence parameter measurements (BP_{ND}). Indeed, some studies have reported morphometric brain abnormalities (Thompson et al., 2004; Chang et al., 2007) in chronic MA users. Although we did find a slightly 4% (nonsignificant) larger striatal volume (ml: MA users, DC, 2.9 ± 0.5; DP, 7.1 ± 0.9; VS, 2.4 ± 0.3; controls, DC, 3.0 ± 0.7; DP, 6.7 ± 1.2; VS, 2.3 ± 0.5) in MA users (no intergroup difference was noted in the occipital cortex: ml MA users, 19.9 ± 2.6; controls, 19.9 ± 2.5) this difference was not greater in short-term abstainers (1–3 d) and is unlikely to explain increased (+)[11C]DTBZ binding. Furthermore, larger striatal volume in MA users cannot account for the results of our voxelwise search. Between-group differences in ROI volumes suggests that partial volume effect (PVE), a bias in estimates of regional radioactivity concentration resulting from spill-in and spill-over artifacts, could differently affect parameter measurements in MA users and controls. However, correction for PVE (geometric transfer matrix) (Rousset et al., 1998) increased (+)[11C]DTBZ BP_{ND} equally in control subjects (VS, 10%; DP, 6%; DC, 45%) and MA users (VS, 10%; DP, 4%; DC, 41%) resulting again in greater striatal (+)[11C]DTBZ BP_{ND} in the MA users relative to controls (+20%, DC; +10%, DP; +11%, VS).

Increased (+)[11C]DTBZ binding might also be explained by upregulation of VMAT2 protein secondary to drug use. However, there appears to be little support for this possibility in the literature with the exception of a single study reporting increased striatal VMAT2 in cocaine (but not D-amphetamine)-exposed rats (Schwartz et al., 2007). In fact, extensive animal data have argued against dopaminergic drug-induced up or downregulation of VMAT2 (Frey et al., 1997; Fleckenstein et al., 2000). Nevertheless, given this preclinical finding in cocaine-exposed animals, and the past use of cocaine by many (10 of 16) of the MA users, this possibility has to be considered.

We propose that striatal binding of (+)[11C]DTBZ (at low radiotracer concentrations in PET studies) is sensitive to endogenous DA (i.e., intravesicular DA and (+)[11C]DTBZ are both competing for the same site on VMAT2) and that vesicular DA concentration in brain of MA users can be low, thereby increasing apparent affinity. In this regard, amphetamines are known to release monoamines from intracytoplasmic vesicles (Sulzer et al., 2005) and therefore would be expected to cause depletion of DA in higher doses. In postmortem brain of MA users, we previously reported that striatal (especially caudate) DA levels can be very low, almost in the parkinsonian range, suggesting that release of DA can be so substantial that vesicular DA stores are depleted until new stores are synthesized (Wilson et al., 1996b; Moszczynska et al., 2004).

Our observation that increased (+)[11C]DTBZ binding occurred in short-term but not long-term abstainers is consistent with "acute" DA depletion followed by partial replenishment. However, this issue can only be addressed in a longitudinal within-subject study. Furthermore, the short-term abstainers were "heavier" than the other MA users in terms of days MA used/last month (8 vs 3), craving and severity of dependence, and possibly had other different characteristics (e.g., persistent impairment in DA biosynthesis) that could have influenced the extent of (+)[11C]DTBZ-binding changes.

The DA–(+)[11C]DTBZ competition argument has previously been used (De La Fuente-Fernández et al., 2003) to explain the PET finding of modestly increased striatal [11C]DTBZ binding in patients with dopa-responsive dystonia (DRD). DRD is a rare hereditary metabolic disorder caused by a deficiency of a DA biosynthetic enzyme and characterized by a brain DA depletion without neuronal loss. Compatible with this view, recent data from our laboratory show that *ex vivo* striatal binding of (+)[11C]DTBZ (administered intravenously at a low tracer dose as in our human PET study) in rats is modestly increased (14 and 12%, respectively) after DA-depleting treatments with either α-methyl-p-tyrosine methylester (3 × 100 mg/kg every 2 h) or D-amphetamine (20 mg/kg) (Tong et al., 2008). Although the above considerations suggest that (+)[11C]DTBZ satisfies a main requirement of the competition model by showing its dependence on endogenous DA, there are many practical uncertainties with the model. The low affinity of DA for the (+)[11C]DTBZ-binding site (>100 μM) would appear to argue against the possibility of decreased DA concentration influencing VMAT2 binding or against a simple competitive interaction between DA and (+)[11C]DTBZ (Partilla et al., 2006); however, vesicular concentration of DA is presumably high (De La Fuente-Fernández et al., 2003). Finally, "non"-competitive interactions between DA and (+)[11C]DTBZ (affecting B_{max}), such as intraneuronal redistribution of VMAT2 (Riddle et al., 2007) or MA-induced changes in concentration of VMAT2-containing vesicles (by unknown mechanism), cannot be excluded.

Is VMAT2 a stable marker of DA neuron integrity in human brain?

Clearly, VMAT2 is a "valid" marker of nigrostriatal DA neuron integrity as indicated by findings of low [11C]DTBZ binding in living (Frey et al., 1996; Lee et al., 2000; this study) and postmortem brain of patients with Parkinson's disease (Wilson et al., 1996a) and in animal models of DA neuron damage (Vander Borght et al., 1995a; Strome et al., 2006; Sossi et al., 2007). However, our PET data in MA users, together with similar findings in DRD (De La Fuente-Fernández et al., 2003), and supported by our new animal data (Tong et al., 2008), suggest that (+)[11C]DTBZ binding, as determined by PET, may be influenced (apart from any actual change in DA neuropil concentration) by changes in vesicular DA levels. If correct, a finding of "normal" striatal (+)[11C]DTBZ binding in MA users might not necessarily indicate preservation of striatal DA innervation and, similarly, a finding of slightly decreased (+)[11C]DTBZ binding in a very heavy drug user might underestimate the extent of neuronal loss. The time course of such a "confound" cannot be determined from our cross-sectional study and might well extend into late abstinence should a DA deficit persist (e.g., attributable to decreased biosynthesis) (Wilson et al., 1996a). Along these lines, a similar argument can be made that striatal (+)[11C]DTBZ binding might typically underestimate the actual magnitude of DA neuron loss in Parkinson's disease, in view of the report of decreased DA biosynthetic capacity in the surviving DA neurons (Kastner et al., 1993).

Striatal (+)[11C]DTBZ PET as an index of vesicular dopamine changes?

On the basis of their PET data in DRD, De La Fuente-Fernández et al. (2003) proposed that "[11C]DTBZ PET might be a useful tool to detect dynamic changes in vesicular DA levels." This suggestion has not received enthusiasm in the scientific community, likely in part because the imaging findings were derived from a small number of subjects having a rare human brain condition and because of the prevailing assumption that VMAT2 is a stable marker. Our new PET data in human MA users support their suggestion, i.e., that [11C]DTBZ PET might be sensitive to decreased vesicular DA. Furthermore, our (+)[11C]DTBZ data in living-brain of drug users also support our early suggestion based on postmortem data that some MA users are exposed to a concentration of the drug sufficiently high to cause depletion of (vesicular) stores of DA. In future studies, comparison in drug users between the extent of vesicular DA depletion as inferred from (+)[11C]DTBZ increase and behavior (e.g., drug craving, anhedonia, relapse) could be helpful in understanding aspects of addiction to psychostimulant drugs. Further information on the rate of recovery over time of DA stores might help guide clinicians as to the intensity and timing of cognitive behavioral interventions in the recently abstinent MA user.

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